

THE FUNCTION OF THE NUCLEUS OF THE LIVING CELL

BY
VERNON LYNCH

A DISSERTATION

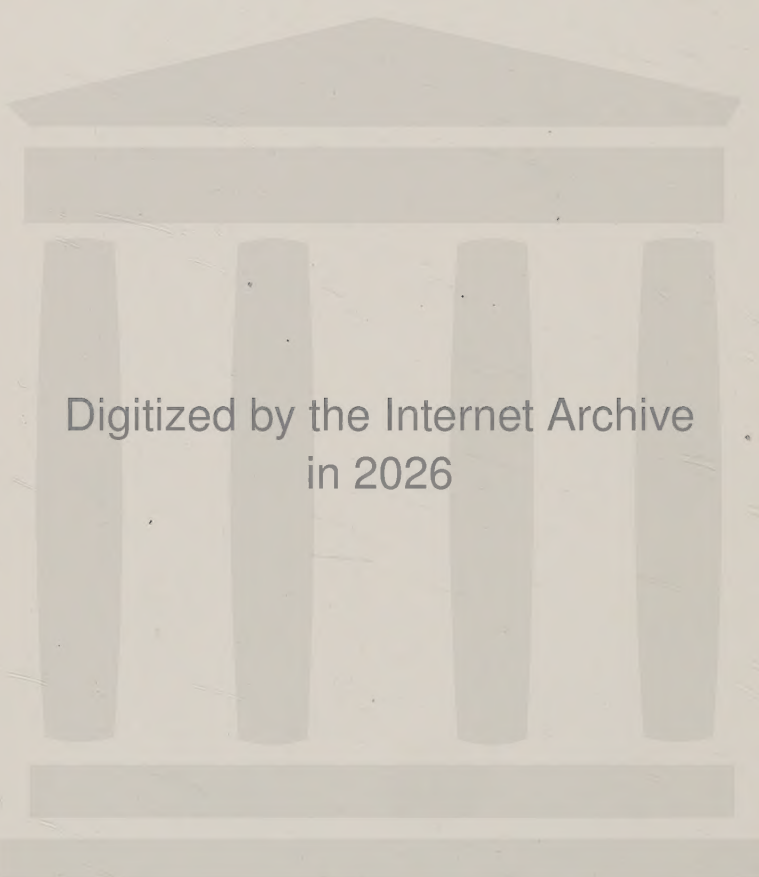
Submitted to the Board of University Studies of the Johns Hopkins
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THE FUNCTION OF THE NUCLEUS OF THE LIVING CELL

VERNON LYNCH

From the Physiological Laboratory of the Johns Hopkins University

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METHOD

Of the different methods which have been employed to solve the problem of the function of the cell nucleus, the one which has given the most illuminating results is the method of extirpation: removing the nucleus and comparing the enucleated cell with a normal nucleated cell. In order that the nucleus may be removed from a cell or that a cell may be cut in half, it is necessary or at least expedient that a cell of considerable size be selected; but at the same time it must be a cell which can be kept alive or cultivated for several generations, if necessary. The cells which meet these requirements most exactly are certain unicellular organisms, certain protozoa. Among such a large and diverse group of organisms it is not difficult to find one which is peculiarly suited for the problem at hand. For the experiments which follow, *Ameba proteus* was selected. Owing to the slow movements and absence of shell in this organism, it can readily be cut in half; or if desired the nucleus may, after some practice, be removed with as little as one-tenth or less of the cytoplasm. Moreover, the lack of differentiation in *Ameba* is a decided advantage for we are certain that in removing part of the cytoplasm with the nucleus we are removing no important organ of the cell. The one disadvantage in the use of this organism is the difficulty of cultivating it. Like all rhizopods, amebas are difficult to raise; but many investigators have succeeded in keeping healthy cultures in the laboratory for years.

In view of the difficulties which are so often met in cultivating amebas, it may not be out of place to add one more method to those which have been described for rearing these valuable experimental animals. It is almost literally true that each investigator has a method of raising amebas which no one can use except himself. This is not surprising when it is seen that "water" is employed in making the cultures, with

little regard for its precise character. While amebas may flourish in the water of one locality, they may quickly die in the water of another locality. This difficulty was avoided by the use of distilled water.¹ The method which follows is based upon one which has been employed by Miss Hyman of the University of Chicago.

A small amount of hay, including both stalks and leaves, is cut into pieces about three inches long; 4 gm. of this material are then placed in a large beaker. To this is added 0.2 gram of dry bread crumbs and 500 cc. of distilled water. The material is boiled for a few minutes and then poured into flat dishes to a depth of 1 to 2 cm. The dishes are covered to keep out dust, and if water evaporates from them it can be replaced by distilled water. After the infusion has cooled, several pipettes full of fluid are added from a culture which contains healthy amebas or from a pond or stream where amebas are found. In doing this care is taken to avoid taking up any of the sediment, for it is the sediment rather than the supernatant fluid which is apt to contain enemies to *Ameba*. This inoculation may be repeated. In the course of two weeks the culture becomes cloudy with bacteria, zoöglea and small ciliates and flagellates, the food of *Ameba*. A small dish of the culture fluid is now seeded with amebas, care being taken to avoid large organisms such as crustacea and worms. If living amebas are not present a week later, more of the fluid containing amebas should be added. This culture is used to inoculate the rest of the infusion. The addition of a little hay occasionally will keep the amebas present in great numbers. The room should not be allowed to become very hot or very cold.

The amebas were transferred to different media by means of a fine glass capillary pipette, the sharp end of which had been made smooth by passing it through a flame. In most of the experiments the animals were kept in drops of fluid upon slides with a depression at each end. The slides were kept in a moist chamber in a room whose temperature did not vary greatly from 20°C. For cutting the amebas in half or cutting the nucleus away from the rest of the cell, a fine glass needle was drawn off at a slight angle from the end of a glass rod. The cells were divided under a binocular microscope.

Since the animals would not live long in the tap water of this city, a search was made at the outset for a solution in which they could be kept in healthy, active condition. Ten amebas were removed from a cul-

¹ The prejudice of many biologists against the use of distilled water and the prevalent notion that it is toxic, are not supported by experiments upon the toxicity of distilled water (Daniel, '08).

Ameba, it has been impossible to get this protoplasm to take in food, and even that food which is already included in the protoplasm is but imperfectly digested. Consequently it is fairer to compare the survival of an enucleated cell with one which has been isolated without food rather than with an actively feeding cell. When this is done it is found that the non-nucleated cell lives almost as long as the nucleated one, *provided there is no difference in size*. If an ameba is cut into unequal parts, the nucleus, owing to its central position, will usually be found in the larger part. Since large fragments live longer than small ones, special care must be taken to avoid a difference in size.

MEDIUM	SURVIVAL OF NUCLEATED PART	SURVIVAL OF NON-NUCLEATED PART
	<i>days</i>	<i>days</i>
Distilled water.....	7	6
Distilled water.....	7	7
Distilled water.....	10	6
Distilled water.....	9	4
Distilled water.....	7	4
Distilled water.....	5	3
Distilled water.....	9	6
Culture water.....	7	5
Culture water.....	8	6
Culture water.....	6	6
Culture water.....	9	7
Culture water.....	11	8
Culture water.....	7	8
Culture water.....	7	7
Culture water.....	7	7
Average.....	7.7	6

Fifteen amebas were cut in half and both halves were kept without food in either distilled water or in water taken from the culture. The number of days which each piece lived after the operation is given in the above table.

It will be noted that in some cases the non-nucleated fragments lived as long as those which contained nuclei. In fact by cutting the ameba in such a way that the piece containing the nucleus was smaller than the piece which lacked it, the non-nucleated piece could be made to outlive the nucleated one. Thus ten amebas were divided in such a way that the nucleated piece was from one-half to one-fourth the size of the other piece. The results are tabulated below:

SURVIVAL OF NUCLEATED PART		SURVIVAL OF NON-NUCLEATED PART	
	<i>days</i>		<i>days</i>
	6		10
	7		7
	3		13
	6		5
	3		6
	6		7
	7		7
	6		8
	7		7
	6		10
Average	5.7		8

We may conclude that a cell deprived of its nucleus may survive as long as a cell deprived of food.

THE NUTRITION OF AMEBA; THE USE OF SUBSTANCES IN SOLUTION

The apparent resemblance of enucleated amebas to amebas deprived of food suggested that some of the phenomena exhibited by the non-nucleated cell might be the result of starvation and have no direct connection with the absence of a nucleus. Accordingly, an attempt was made to supply the amebas with adequate food substances. Since it was not possible to get the enucleated organisms to take in food particles, an effort was made to provide a medium in which amebas could be nourished by the absorption of substances in solution.

It has been pointed out (Burrows and Neymann, '17) that no artificial synthetic medium has ever been provided which was adequate to nourish an animal cell although the blood and lymph apparently constitute a natural synthetic medium for the cells of higher organisms. Even cells cultivated in vitro depend upon the autolysis of neighboring cells for their nourishment, and the attempt to cultivate protozoa in nutritive organic media has met with complete failure (Biedermann, '16; Doflein, '11). In the light of recent experiments in nutrition, however, it seems possible that such negative results may be due to the failure to supply certain substances which are necessary for proper nutrition. An attempt was therefore made to provide an adequate medium for the primitive animal cell, *Ameba proteus*.

Since amebas were found to move more normally and to live longer in

distilled water to which had been added one-tenth the volume of spring water, this "10 per cent spring water" was used in making all solutions. Various carbon compounds, including several sugars, were now tested with the hope of finding a source of energy.

DATE	NUMBER OF AMEBAS LIVING		
	Water	1 per cent glucose	1 per cent levulose
February 7.....	20	20	20
February 8.....	20	20	20
February 9.....	18	20	20
February 10.....	15	20	20
February 11.....	15	20	20
February 12.....	15	20	20
February 13.....	13	20	20
February 14.....	13	20	20
February 15.....	13	20	20
February 16.....	13	20	19
February 17.....	13	20	19
February 18.....	13	20	18
February 19.....	13	20	18
February 20.....	11	18	17
February 21.....	11	17	17
February 22.....	11	17	15
February 23.....	9	17	15
February 24.....	7	17	15
February 25.....	6	16	14
February 26.....	6	16	12
February 27.....	4	12	11
February 28.....	3	12	8
March 1.....	2	11	8
March 2.....	2	10	2
March 3.....	2	6	0
March 4.....	1	4	
March 5.....	1	3	
March 6.....	1	0	
March 7.....	1		
March 8.....	0		
Average life.....	13.6 days	21.8 days	19.4 days

The method used was as follows: A number of amebas were selected from the same culture and freed from food and bacteria by transferring repeatedly to fresh solutions of 10 per cent spring water. They were then transferred to slides, placing ten in 10 per cent spring water

as a control, and ten in each of the solutions to be tested. The animals were examined and counted each day, washed in water and transferred to fresh solutions to prevent the development of bacteria. The most promising substances found were the hexoses, glucose and levulose, the effect of which may be seen in the preceding experiment.

DATE	NUMBER OF AMEBAS LIVING		
	1 per cent glucose	1 per cent glucose + 0.1 per cent urea	1 per cent glucose + 0.01 per cent NH_4NO_3
February 24.....	10	10	10
February 25.....	9	10	8
February 26.....	9	11*	8
February 27.....	9	12	8
February 28.....	6	12	8
March 1.....	5	12	8
March 2.....	5	13	8
March 3.....	4	12	8
March 4.....	4	12	8
March 5.....	4	12	8
March 6.....	4	10	8
March 7.....	4	10	8
March 8.....	4	10	8
March 9.....	4	8	7
March 10.....	4	6	7
March 11.....	4	6	7
March 12.....	4	6	4
March 13.....	3	5	3
March 14.....	2	4	2
March 15.....	1	3	2
March 16.....	1	3	1
March 17.....	1	3	0
March 21.....	0	1	
March 22.....		1	
March 27.....		0	
Average life.....	10.4 days	18.9 days	13.9 days

*The increase in number shown in this and subsequent tables is the result of the division of certain of the amebas.

Not only did the amebas live longer in the sugar solutions but during the early part of the experiment they were much more active. At the end of a week, however, many of the amebas began to take on an opaque appearance and ceased normal movements. The substance which was

formerly a food now acted as a poison. Child ('15) has shown that susceptibility to poisons increases during starvation.

Although these cultures were not sterile, it was possible to keep the bacteria from becoming numerous and thus prevent their becoming an appreciable source of food for the amebas.

Having found a carbohydrate food for Ameba, a search was now made for a source of nitrogen. In such a primitive cell the possibility that simple compounds of nitrogen may be used at once suggests itself. Of various compounds of nitrogen which were tried, the best results were obtained with ammonium nitrate and urea; and of these two, urea was much the better owing to its comparatively low toxicity. The effect of these substances is seen in the preceding experiment. (The comparatively short life of the animals is to be attributed to the use of an inferior culture.)

That these compounds are of some use to the amebas is indicated by the increased length of life when they are added to the solution; but that they are not an adequate source of nitrogen is indicated by the fact that the animals eventually die and that no growth can be detected. The shrinkage caused by the glucose would render the detection of growth difficult even if present.

Of special interest, however, are the three cases of cell division in the urea solution. Although hundreds of amebas have been kept under daily observation until they were completely disintegrated, no single case of cell division was ever observed unless food substances were supplied; and with two possible exceptions, no division was observed unless a source of nitrogen was added. On the other hand, divisions have been repeatedly observed in solutions containing urea or certain amino acids. That the division is not simply the result of the stimulating action of the urea upon the protoplasm is shown by the effect of urea solutions to which no glucose is added. In such solutions the amebas not only do not divide, but they die more quickly than in water alone. Urea in the absence of glucose is simply a mild poison but when urea and glucose are used together, the animals live longer, have a more normal appearance and may even reproduce. The necessity of using the two substances together indicates that they are built up or combined to form some more complex substance which is of use to the organism.

If this is a true case of organic synthesis, it would be interesting to see whether it occurs in the cell which has been deprived of its nucleus, in order to throw light upon the supposed synthetic function of the nucleus. This point is taken up later.

Having failed to obtain growth with simple compounds of nitrogen, the organisms were now supplied with amino acids. The use of simple amino acids was not promising. Some reproduction was obtained with a saturated solution of tyrosin, but the animals soon died. A mixture of amino acids was then prepared in the following way:

Five grams of Hammarsten's casein was heated for twenty hours with 100 cc. of a molecular solution of sulphuric acid. The hydrolysis was performed on a water bath in a flask fitted with a reflux condenser. At the end of the hydrolysis, when the solution no longer gave the biuret reaction, a saturated solution of barium hydroxide was added until the reaction was slightly alkaline, and the solution boiled to remove ammonia. Dilute sulphuric acid was added until the reaction was very slightly acid, and the solution filtered hot. The barium sulphate precipitate, which had absorbed much of the humus substance, was not washed. The solution was exactly neutralized with sodium hydroxide, and the resulting fluid, whose volume was 700 cc., was clear and straw-colored and had a bitter-sweet taste. The reactions for barium, calcium, iron, reduced sulphur and tryptophane were negative; phosphate was present and the xantho-proteic reaction was positive. When exposed to the air, bacteria developed with remarkable rapidity. The solution was not toxic to amebas when evaporated to one-half the volume, but evaporation to one-fifth the volume resulted in a solution which was slightly toxic.²

This solution of amino acids lacks glycine, which is absent in casein, and tryptophane and cystine, which were destroyed.

In cultivating amebas in this solution it was difficult to prevent the development of bacteria, but a method was devised which served to exclude them almost completely. The amebas were washed five to ten times in sterile water, and the slides upon which they were kept were boiled. The culture solutions were kept in small flasks fitted with capillary tubes. By inverting the flask and warming with the hand, a few drops could be placed upon the slide. After using, the flask was boiled and a test tube placed over its neck. The cultures were examined daily, the amebas washed in sterile water and transferred to new solutions.

The hydrolyzed casein solution proved to be a fairly good medium in which cases of cell division were occasionally observed, but there was

² The toxicity of amino acids observed by Burrows and Neymann ('17) upon embryonic chicken cells was probably due to the relatively concentrated solutions used.

never any growth and the changes which preceded death could be seen in about ten days. The addition of glucose resulted in some improvement but it did not prevent death.

DATE	NUMBER OF AMEBAS LIVING	
	Hydrolyzed casein	Hydrolyzed casein + 0.2 per cent glucose
May 1.....	5	5
May 2.....	5	6*
May 3.....	6*	6
May 4.....	6	6
May 5.....	6	7
May 6.....	6	8
May 7.....	6	8
May 8.....	Dying	8
May 9.....	Discontinued	8
May 10.....		8
May 11.....		8
May 12.....		8 Amebas healthy and active
May 13.....		8 Amebas somewhat abnormal
May 14.....		7
		Dying. Experiment discontinued

* See footnote to table, p. 14.

DATE	NUMBER OF AMEBAS LIVING
	Hydrolyzed casein + 1 per cent glucose + 0.1 per cent tryptophane + 0.03 per cent cystine + 0.2 per cent milk.
May 23.....	5
May 24.....	5
May 25.....	6*
May 26.....	6
May 27.....	6
May 28.....	6
May 29.....	6
May 30.....	6
May 31.....	6
June 1.....	6
June 2.....	6
June 3.....	5
June 4.....	3
June 5.....	3
June 6.....	1
June 7.....	0

* See footnote to table p. 14.

Since this death might be due to the absence of some foodstuff, various substances were added to the "diet." The addition of various salts was tried but no improvement was observed. Cystine and tryptophane, so important in the nutrition of higher animals, were added to the solution in concentrations which were shown not to be toxic. A small amount of milk was added to provide the unknown "accessory" food substances, and also certain salts (see lower table on page 17).

No synthetic medium was found for *Ameba proteus*. The failure may be due to some peculiarity of the cell, such as its enormous size, or to a low permeability.

THE NUTRITION OF THE NON-NUCLEATED CELL

Since, as was shown above, glucose prolongs the life of amebas and apparently acts as a food, it would be interesting to know whether it has a similar effect upon the cell from which the nucleus has been removed. To determine this, the nucleus was removed from a number of amebas, care being taken to remove as little cytoplasm as possible. Under favorable circumstances it is possible to cut the nucleus from the cell without removing more than one-tenth of the cytoplasm. Some of the enucleated amebas were now transferred to water while an equal number of others of equal size were kept in glucose solutions. The usual precautions were taken to prevent the development of bacteria.

DATE	NUMBER OF AMEBAS LIVING	
	Water	0.5 per cent glucose
December 3.....	9	9
December 4.....	9	9
December 5.....	9	9
December 6.....	9	9
December 7.....	9	9
December 8.....	9	9
December 9.....	9	9
December 10.....	9	9
December 11.....	8	8
December 12.....	4	7
December 13.....	3	6
December 14.....	2	4
December 15.....	0	3
December 16.....		1
December 17.....		0
Average life.....	9.9 days	11.2 days

Although the amebas kept in glucose lived somewhat longer than the controls, the effect was not very striking. Since there are great differences between different amebas, even when selected from the same culture, a somewhat more satisfactory experiment was performed as follows: The nucleus was removed from a number of amebas and then the amebas were divided in half. One-half was placed in water, as a control, while the other half was kept in a solution of glucose.

DATE	NUMBER OF AMEBAS LIVING	
	Water	1 per cent glucose
April 3.....	12	12
April 4.....	12	12
April 5.....	12	12
April 6.....	12	12
April 7.....	11	11
April 8.....	10	11
April 9.....	9	11
April 10.....	6	10
April 11.....	3	6
April 12.....	2	4
April 13.....	0	3
April 14.....		0
Average life.....	7.4 days	8.7 days

DATE	NUMBER OF AMEBAS LIVING		
	Glucose	Glucose + 0.1 per cent urea	Glucose + 0.01 per cent NH_4NO_3
November 19.....	10	10	9
November 20.....	10	10	9
November 21.....	10	10	9
November 22.....	10	9	9
November 23.....	10	9	8
November 24.....	10	7	8
November 25.....	10	5	8
November 26.....	9	2	7
November 27.....	6	1	5
November 28.....	5	0	2
November 29.....	4		2
December 2.....	0		1
December 3.....			0
Average life.....	10.2 days	6.3 days	9.0 days

The non-nucleated cells live longer in glucose solutions and probably use it as a food. This is in agreement with the work of Rous and Turner ('15), who used glucose for the preservation of the non-nucleated red blood corpuscles.

If the life of the non-nucleated cell is prolonged by the use of glucose, is it further prolonged by the use of urea or ammonium nitrate?

The nucleus was removed from twenty-nine amebas, and after washing, ten were placed in glucose, ten in glucose plus urea, and nine in glucose plus ammonium nitrate (see lower table on page 19)

Thus in the non-nucleated cell, the addition of urea to the glucose was not beneficial; on the contrary it was harmful. The addition of the ammonium nitrate was without effect.

Evidence was offered above that the normal, nucleated ameba was able to form some combination between glucose and urea (or some derivative of urea). If it is true that the beneficial effect of glucose plus urea depends upon a synthesis, we may conclude that the non-nucleated cell is unable to perform this synthesis.

RESPIRATION IN THE NON-NUCLEATED CELL

It has been suggested (Loeb, '05) that the nucleus is the organ of oxidation of the living cell and that peculiarities of the non-nucleated cell, such as lack of the power to synthesize or to regenerate lost parts, are the result of the lowered oxidative activity. The non-nucleated pieces, it is said, "die slowly from asphyxia."

If this theory is true, we should expect to find a marked difference between the effect of depriving the non-nucleated cell of oxygen and the effect of depriving the nucleated cell of oxygen. We should expect the cell in which oxidations were occurring most rapidly to be most affected by its removal, whereas the cell which was using very little oxygen should not be greatly affected. According to Child ('15), regions in which respiration is rapid are more susceptible to lack of oxygen and die sooner than regions in which respiration is slower.

The effect of depriving the nucleated and non-nucleated cells of oxygen was investigated in the following manner: Five or more amebas were divided into two pieces of as nearly equal size as possible. After leaving them for a longer or shorter period in air, they were placed in hanging drops, side by side, in an Engelmann gas-chamber. Nitrogen, which had been washed in water, was then passed through the chamber, and this atmosphere was maintained until the death of the organisms.

In the third experiment the nitrogen was first bubbled from a capillary tube through strongly alkaline pyrogallie acid, to remove possible traces of oxygen. In the first experiment, the temperature of the water jacket surrounding the chamber was raised to 26°C. in order to accelerate the experiment. The other experiments were performed at 20°C.

Experiment 1

TIME	NUMBER OF AMEBAS LIVING	
	Nucleated	Non-nucleated
5.00 p.m.....	Amebas divided	
9.50 a.m.....	Placed in nitrogen	
10.00 a.m.....	7	7
11.00 a.m.....	7	7
12.00 a.m.....	7	6
1.00 p.m.....	7	4
1.20 p.m.....	7	3
1.30 p.m.....	6	1
2.00 p.m.....	4	0
2.25 p.m.....	3	
2.30 p.m.....	2	
2.35 p.m.....	1	
2.45 p.m.....	0	
Average life.....	4.4 hours	3.4 hours

Thus there is a distinct difference in the susceptibility to lack of oxygen in the nucleated and non-nucleated cell, but this difference is just the opposite to what we should expect if oxidations were proceeding more rapidly in the nucleated half. Not only does the non-nucleated cell die more quickly when deprived of oxygen, but it is the first to assume a spherical shape and to cease putting out pseudopods.

The opposite experiment, that of increasing the supply of oxygen, is also of interest. Were it true that in removing the nucleus we have removed the organ of oxidation, and that the cell is slowly dying of asphyxia, it should be possible to delay the death of the non-nucleated cell by supplying it with more oxygen. On the contrary, it was found that when the nucleated and non-nucleated halves of amebas were kept in an atmosphere of oxygen, both died in less than twelve hours and the non-nucleated pieces were killed quite as rapidly as those which contained nuclei.

Experiment 2

TIME	NUMBER OF AMEBAS LIVING	
	Nucleated	Non-nucleated
10.30 a.m.....	Amebas divided	
11.05 a.m.....	Placed in nitrogen	
12.00 a.m.....	5	5
1.00 p.m.....	5	5
2.00 p.m.....	5	5
3.00 p.m.....	5	5
4.00 p.m.....	5	5
5.00 p.m.....	5	5
6.00 p.m.....	5	5
7.00 p.m.....	5	4
7.30 p.m.....	5	2
8.30 p.m.....	4	2
9.00 p.m.....	4	2
10.00 p.m.....	4	1
11.00 p.m.....	4	1
12.00 p.m.....	4	1
8.00 a.m.....	3	1
9.00 a.m.....	3	1
9.30 a.m.....	3	0
10.00 a.m.....	3	
10.15 a.m.....	1	
11.00 a.m.....	1	
12.00 a.m.....	1	
1.00 p.m.....	1	
2.00 p.m.....	0	
Average life.....	20.5 hours	11.6 hours

Experiment 3

TIME	NUMBER OF AMEBAS LIVING	
	Nucleated	Non-nucleated
9.00 a.m.....	Amebas divided	
10.20 a.m.....	Placed in nitrogen	
11.00 a.m.....	5	5
12.00 a.m.....	5	5
1.00 p.m.....	5	5
3.00 p.m.....	5	4
4.00 p.m.....	5	2
5.00 p.m.....	5	2
8.00 p.m.....	5	1
9.00 p.m.....	4	1
9.30 p.m.....	4	0
10.00 p.m.....	2	
11.00 p.m.....	2	
12.00 p.m.....	2	
6.00 a.m.....	0	
Average life.....	14.6 hours	7.4 hours

The experiment was performed in the following manner: Five amebas were cut in half with a fine glass needle, and each half was transferred to a hanging drop in a gas chamber. Oxygen which had been thoroughly washed was now passed through the chamber and the organisms kept under observation until all had disintegrated. The animals were divided at 10 a.m. Oxygen was passed through at 10.30 a.m.

Time of death

NUCLEATED CELL	NON-NUCLEATED CELL
1.30	2.00
2.00	2.10
4.15	3.23
5.00	3.27
6.30	4.15
Average life... 5.4 hours	4.6 hours

The injurious action of oxygen, which is doubtless the result of an increase in the oxidations of the cell, took effect somewhat more rapidly upon the cell which lacked a nucleus. It is difficult to see how a cell could be killed in a few hours by an atmosphere of oxygen if its oxidations have become depressed.

THE EFFECT OF TEMPERATURE UPON THE NON-NUCLEATED CELL

In the experiments hitherto reported, a fairly constant temperature of 20°C. had been maintained. An attempt was now made to determine the effect of temperature upon the nucleated and non-nucleated cells, with the hope of discovering a possible difference in the rate at which chemical changes were occurring in the two cells. Two methods of investigation suggest themselves:

1. The temperature of the medium may be gradually raised until death occurs.
2. The organisms may be kept at a rather high temperature and observed until death occurs.

Both of these methods were used in the order named.

In the first method amebas were placed upon a Pfeiffer warming stage, through which water of any desired temperature could be passed. After dividing the ameba into two equal parts, the temperature of the warming stage was raised at the rate of about one degree Centigrade

per minute. The rate at which the temperature is raised is of importance for by raising the temperature rapidly the organisms may be killed at a comparatively low temperature. As the temperature rises, the amebas soon withdraw all of their pseudopods and assume a spherical shape. This does not, however, indicate the death of the organism for if at this point the temperature is slowly lowered to 20°C. the animals will, in a few hours, send out pseudopods and resume normal movement. If the temperature is raised high enough, the cells will either disintegrate completely or coagulate, with the result that the cell boundary can no longer be seen. The temperature at which the spherical shape was assumed and the temperature at which death occurred are recorded below.

EXPERIMENT	SPHERICAL SHAPE ASSUMED		DEATH	
	Nucleated	Non-nucleated	Nucleated	Non-nucleated
	°C.	°C.	°C.	°C.
1	39	38	51	50
2	40	39	50	50+
3	40	40	47	52
4	39	44	49	48
Average.....	39.5	40.2	49.2	50.1

The effect of gradual cooling was not so easily determined. When the temperature of the fluid was lowered greatly and even under-cooled, there was no injury provided crystallization did not take place. When the solution crystallized, however, as the result of adding a small crystal of ice, the amebas were injured mechanically and disintegrated as soon as the crystals melted.

But the effect of cooling could be shown in a somewhat different manner. The nucleated and non-nucleated parts of an ameba were slowly warmed to 44°C. and then cooled in ten minutes to 22°C. Two minutes later both fragments disintegrated at the same time, the result of the rapid cooling.

Thus no difference could be found in the susceptibility of the nuclear and non-nuclear fragments to a rise or fall of temperature. The other method was now employed: keeping amebas at different temperatures until death occurred.

Eleven amebas were freed from food particles and debris and divided into two equal parts. Both the nuclear and non-nuclear fragments were kept in a moist chamber at an average room temperature of 20°C.

Experiment 1. 20°C.

DATE	NUMBER OF AMEBAS LIVING	
	Nucleated	Non-nucleated
October 10.....	11	11
October 11.....	11	11
October 12.....	11	11
October 13.....	8	10
October 14.....	7	6
October 15, a.m.....	7	3
October 15, p.m.....	5	2
October 16.....	4	2
October 17.....	4	2
October 18.....	4	2
October 19, a.m.....	3	1
October 19, p.m.....	1	0
October 20.....	0	
Average life.....	6.2 days	5.3 days

Thus, as was shown before, the life of the non-nuclear fragment at ordinary temperature is almost as long as that of the nuclear fragment.

Ten amebas were now divided and each placed in a moist chamber which was kept in a thermostat at 30°C.

Experiment 2. 30°C.

DATE	NUMBER OF AMEBAS LIVING	
	Nucleated	Non-nucleated
October 22.....	10	10
October 23, a.m.....	10	7
October 23, p.m.....	9	7
October 24.....	9	4
October 25.....	9	2
October 26, a.m.....	6	1
October 26, p.m.....	5	0
October 27.....	3	
October 29.....	3	
October 30.....	3	
October 31.....	1	
November 1.....	0	
Average life.....	6.0 days	2.4 days

Apparently the life of the nuclear fragments was not shortened by exposure to high temperature, but this was probably due to the use of healthier amebas in experiment 2. Further experiments showed that the life of nucleated amebas was shortened by raising the temperature but the life of non-nuclear amebas was shortened somewhat more.

Twelve amebas were now divided and placed in a moist chamber at 10°C. The moist chamber was allowed to warm slowly to room temperature before removing the slide for examination.

Experiment 3. 10°C.

DATE	NUMBER OF AMEBAS LIVING	
	Nucleated	Non-nucleated
October 17.....	12	12
October 18.....	12	12
October 19.....	12	12
October 20.....	12	12
October 21.....	12	12
October 22.....	12	12
October 23.....	11	11
October 24.....	11	11
October 25.....	11	11
October 26.....	11	11
October 27.....	11	11
October 29, a.m.....	11	9
October 29, p.m.....	11	8
October 30, a.m.....	11	5
October 30, p.m.....	11	4
October 31, a.m.....	11	3
October 31, p.m.....	10	2
November 1, a.m.....	5	0
November 1, p.m.....	3	
November 2.....	0	
Average life.....	14.5 days	12.8 days

The life of both fragments was prolonged by cold in this case but in two other cases it was distinctly shortened. This may have been due to the use of different cultures.

If susceptibility to high or low temperature may be used as an index to the rate of metabolism, there is no very great difference between the two fragments. There is some evidence that cells in which metabolic processes are rapid show a greater susceptibility to high temperature

ture, freed from food material and debris and transferred to the solution to be tested. Each day they were examined and transferred to a fresh solution, the experiment being continued until all of the animals were dead. It appeared at first that distilled water was the most satisfactory medium that could be obtained. Thus ten amebas lived (without food) for an average of 13.2 days in distilled water, whereas animals kept in tap water, for example, soon assumed an abnormal appearance and could seldom be kept alive longer than one week. But it was later discovered that the distilled water was greatly improved by the addition of one-tenth the volume of spring water. Not only was the average life ($14\frac{1}{2}$ days) somewhat longer, but the condition of the animals during this time was greatly improved. In the distilled water the animals soon cease to be attached to the bottom and are hence unable to move from place to place in the normal manner; but in the "10 per cent spring water," after it became saturated with air, they remained attached up to a few days before death. The spring water used contained a trace of calcium, but not so much as was contained in the tap water. In the following experiments the amebas, unless otherwise noted, were kept in the 10 per cent spring water.

RELATION OF THE NUCLEUS TO MOVEMENT

The movement of normal amebas in a suitable medium, such as the dilute spring water, when placed upon a glass slide, was found to be of the limax type. The ameba is attached to the slide by means of some sticky secretion and flows steadily forward, sending out pseudopods at the anterior end, often from alternate sides. Frequently amebas are found which are not attached to the slide and such amebas may project pseudopods in any direction, but are unable to move from place to place. These animals will in time, however, adhere to the slide, unless they are abnormal or the medium is unsuitable. When these active amebas are stimulated roughly, as by pressure with a glass needle, they retract into a more or less spherical shape and small droplets of protoplasm may be seen projecting from the surface.

The effect of amputation of the nucleus upon the movement of the cell may be seen from the following typical experiment:

- 3.21 p.m. An active ameba which was attached to the slide was divided into two approximately equal parts, the nucleus remaining in the posterior half. Both parts went into the typical condition of stimulation.

- 3.23 p.m. Both pieces have put out pseudopods and are active, but the non-nucleated part, which was cut from the anterior end, is much more active than the nucleated and posterior part.
- 3.27 p.m. Both pieces are moving in the typical limax fashion, but the movement of the non-nucleated piece has become perceptibly slower.
- 3.28 p.m. The non-nucleated piece ceases its progressive movements and slowly retracts into a somewhat corrugated sphere. Only an occasional, very blunt pseudopod is now produced, and slight agitation shows that the fragment is no longer attached to the slide. The nucleated piece differs from a normal ameba only in size.

The retraction into the spherical shape is an invariable phenomenon and is of such a definite character that the time of its occurrence may be accurately determined. Thus in fifteen amebas which were so divided that the nucleus remained in the posterior half, the retraction occurred at an average of nine minutes after the operation. To the observer it resembles strongly the response to stimulation, except for the absence of the protoplasmic droplets mentioned above. The protoplasm is capable of movement, as is shown by transferring it to another medium such as distilled water, to which it responds by a change of shape.

This effect of amputation of the nucleus upon movement is not always permanent. If a number of amebas are cut in half and the nucleated and non-nucleated pieces observed each day, it will be noticed on the first or second day after the operation that many of the non-nucleated fragments are moving in the typical limax fashion, and in fact there is sometimes no great difference between the movements of the nucleated and the non-nucleated pieces. Slight agitation, however, shows that the non-nuclear amebas are scarcely attached to the slide; the slightest disturbance serves to dislodge them. This fact, which must be due to the failure to produce the sticky secretion, is probably partly responsible for the difference in movement between the nuclear and the non-nuclear parts.

The fact that a non-nucleated ameba may, under any conditions, exhibit normal movements, justifies us in concluding that the nucleus is not necessary for movement. In *Ameba*, however, movement is affected in some indirect way by removal of the nucleus.

THE SURVIVAL OF THE NON-NUCLEATED CELL

All observations made upon cells which have been deprived of their nuclei prove conclusively that non-nucleated protoplasm is destined to die without growing or dividing. But in most cases, particularly in

than do cells with a low metabolism (Child, '15, p. 66). If this is true, there is some slight indication of a more rapid metabolism in the non-nuclear fragment.

METABOLIC RATE IN THE NON-NUCLEATED CELL

The rate at which metabolic processes are occurring in different parts of an organism or tissue has been very successfully studied by determining their susceptibility to various poisons, especially to potassium cyanide (Child, '13, '15). Child has brought together a great deal of direct and indirect evidence to show that, in general, regions of high metabolism are more susceptible to poisons than are regions of low metabolism. Accordingly, the susceptibility of the nucleated and non-nucleated cell was investigated with a view to determining how the rate of metabolism was affected by the removal of the nucleus.

The amebas were cut in half and one hour after the operation they were transferred to a fresh $\frac{M}{50}$ solution of KCN upon a hollow-ground glass slide. The drop of solution was immediately covered with a cover slip to prevent the escape of HCN. Since this could not be entirely prevented, the solution gradually became weaker and as a result those amebas which were not killed in two or three minutes usually lived for more than an hour. Since small fragments of ameba were found to be more susceptible than large ones, an effort was made to divide the amebas into equal parts. After more than fifty Amebas had been treated with cyanide, it was obvious that some unknown factor was playing a part in the death of the organisms; for although the non-nucleated fragments usually succumbed first, there were many conspicuous cases in which the nucleated part died much sooner. It seemed possible that the unknown factor might be the difference between the anterior and posterior end. The amebas were divided when they were moving along the bottom in the "limax" condition, and in some cases the non-nuclear fragment was taken from the flowing anterior end while in other cases it was taken from the retracting posterior end. In order to determine whether this made a difference, a number of amebas were divided in such a way that the nucleus remained in the anterior half, while others were so divided that the nucleus remained in the posterior half.

The number of minutes which the nuclear and non-nuclear fragments were able to withstand treatment with cyanide is tabulated below:

Duration of life

NUCLEUS IN ANTERIOR END		NUCLEUS IN POSTERIOR END	
Nucleated	Non-nucleated	Nucleated	Non-nucleated
<i>min.</i>	<i>min.</i>	<i>min.</i>	<i>min.</i>
96	8	190	83
5	34	155	10
45	15	145	5
200	200	85	1
190	190	170	167
45	170	5	5
260	240	145	115
1	2	146	2
290	530	105	2
580	62	100	1
6	180	98	7
2	140	5	3
260	1	1.5	1
260	25	1.9	0.8
300	15	76	2
230	3	74	2
230	140	6	2
320	305	80	11
87	2	80	10
230	6	75	5
185	41	1	0.5
255	450	12	1
450	250	9	3
2	445	25	25
4	100		
305	1		
10	1		
120	1		
1	0.5		
410	410		
330	330		
320	1		
Average: 188.4	134.3	74.6	19.3

When the nucleus was in the anterior end, the non-nuclear fragment died first in 19 cases, the nuclear fragment died first in 7 cases and in the remaining 4 cases each part disintegrated at about the same time. When the nucleus was in the posterior end, however, the nuclear fragment was never the first to disintegrate; the death of both fragments occurred

at the same time in two instances, and in the remaining 22 cases the non-nuclear fragment was the first to disintegrate. The complicating factor is now understood. The anterior end of *Ameba* is more susceptible to cyanide than the posterior end. Essentially the same conclusion was reached by Miss Hyman ('17) in an investigation of the metabolic gradient in *Ameba*.

When the non-nucleated half is taken from the posterior end, its greater susceptibility to cyanide is not very definite because of the difference which exists between the anterior and posterior parts. When this disturbing factor is removed, however, by cutting the non-nuclear fragment from the anterior end, the non-nuclear fragment is seen to be much more susceptible to cyanide than the nuclear fragment.

The same difference in susceptibility between the nucleated and non-nucleated cells was found when they were kept for twenty hours after the operation before treatment with cyanide.

Whether we can conclude that the rate of metabolism is increased by removal of the nucleus is doubtful. It is possible that removal of the nucleus decreases the resistance to cyanides for some other reason. But, at any rate, the evidence such as it is opposes the view that respiration and metabolism are depressed by removal of the nucleus.

SUMMARY AND CONCLUSIONS

1. An *ameba* from which the nucleus has been removed may at times exhibit perfectly normal movement; in general, however, movement is somewhat affected by removal of the nucleus.

2. An *ameba* deprived of its nucleus lives almost as long as an *ameba* deprived of food.

3. Evidence is offered that *Ameba* can use glucose in solution as a food. There is also evidence that *Ameba* can synthesize glucose and urea, or some derivatives of these substances, to form a product which is of nutritive value.

4. Glucose is also of some benefit to the enucleated *ameba* but the supposed synthesis of glucose and urea can not be performed.

5. The non-nucleated cell is injured more quickly by either a lack or an excess of oxygen than is the normal nucleated cell.

6. The non-nucleated cell is somewhat more susceptible to high or low temperature than the nucleated cell.

7. The non-nucleated cell is more susceptible to cyanide than the nucleated cell.

DISCUSSION

There are two important theories of nuclear function: the theory of *synthesis* and the theory of *oxidation*. According to the former theory, the non-nucleated cell is unable to construct or synthesize new substances; while according to the latter, it is more or less unable to oxidize the substances already present. In reviewing the experiments here reported it is seen that they not only offer strong evidence for the validity of the synthesis theory, but they constitute a practical proof that the oxidation theory is not correct.

From the outset there has been no satisfactory evidence in support of the oxidation theory. It was apparently suggested by some experiments reported by Spitzer ('97). Spitzer obtained from various cells a preparation of nucleoprotein which contained a small amount of iron and which had the property of decomposing hydrogen peroxide. From this it has been concluded not only that all nuclei contain iron, but even that all nucleoproteins contain iron. The latter statement is of course false, and the microchemical evidence in support of the former statement (Macallum, '92) is very unsatisfactory.³ The presence of iron in the nucleus was taken as an indication that the nucleus played an important rôle in oxidations, but the presence of this iron is open to doubt. Spitzer's catalase may have been derived from the cytoplasm.

The chemical evidence for the oxidation theory is very slight and the physiological evidence is equally so. The assumption that the organ which is furthest removed from the supply of oxygen is the organ of oxidation is not probable in the first place. "It should be borne in mind that oxygen, in order to reach the nucleus, must penetrate a layer of cytoplasm containing reducing substances." (R. S. Lillie, '11.) Moreover, as was shown in the above experiments, the non-nucleated cell may exhibit perfectly normal movements. Since the energy for movement in aerobic organisms is derived from the oxidation of organic matter, it is unlikely that oxidations have become depressed. But if there is a depression of oxidations we should certainly expect (cf. Loeb, '05) to find some improvement in the cell when we increase its supply of oxygen. On the contrary, as has been shown, the cell becomes spherical and dies in a few hours. Also the susceptibility to lack of oxygen,

³ From a very pure preparation of the sperm heads of the Great Lakes Whitefish, the author was unable to find a trace of iron in 0.5 gram of the dry material by the sulphocyanide test. The ashing was done in the electric muffle and by Neumann digestion, so that there was no question of "masked" iron. (Unpublished.)

the susceptibility to cyanide and the susceptibility to high and low temperature all indicate that respiration and rate of metabolism are quite as rapid in the enucleated cell as in the normal cell.

Recently, however, experiments have been reported which seem to give strong support to this theory; namely, the experiments of Osterhout on the leaf of the Indian Pipe. When the leaf of this plant is cut, the cells which have been injured soon become dark. Osterhout ('17) has shown that this darkening is an oxidation and since it occurs first in the nucleus, he argues that the nucleus is the center of oxidations. These experiments, however, admit of another interpretation. It is reasonable to believe that at the center of the cell there is a strong reducing action and that very little oxygen is present. After the death of the cell, however, oxygen, like other substances, may enter freely, and in the *dying* cell it would be expected that oxidations would be very rapid in the neighborhood of the nucleus, where the reducing action is greatest.

Moreover, Kite and Chambers ('12) found, in cells which were apparently uninjured, that *reduction* was most rapid in the region of the nucleus. The dye janus green becomes red when reduced. Kite and Chambers observed that this reduction occurred first at the centrosome and second in the chromatin of the nucleus.

The experiments here reported offer support to the theory that the nucleus is the organ of synthesis. When a cell is deprived of food the processes of synthesis must, to a great extent, come to a stop, although synthesis of intermediary products of metabolism is still possible. It is probably not a coincidence that a cell deprived of its nucleus lives almost as long as a cell deprived of food.

An opportunity to put the synthesis theory to a direct test was afforded by nutritional experiments. When amebas are kept without food they will die in from one to two weeks, but if glucose is present in solution their life is somewhat prolonged. It appears that the glucose is used as a food. When urea was added the organisms lived even longer and in some cases divided. Urea alone, without glucose, had no such effect, a fact which is interpreted as meaning that the nitrogen compound is combined by the cell with the carbon compound. When nitrogen was already combined with unoxidized carbon, as in the amino-acids, the addition of glucose was unnecessary.

When these experiments were repeated upon the enucleated cell, it was found that glucose was somewhat beneficial and was apparently used as a food. But the further addition of urea was not only of no

benefit, but actually hastened the death of the protoplasm. Apparently non-nuclear protoplasm is unable to combine carbon and nitrogen: the power of synthesis is absent.⁴

There is further evidence of the loss of the power of synthesis in the failure to produce the sticky secretion by means of which *Ameba* attaches itself to various objects. Normal amebas are firmly attached to the slide and frequently stick to the sides of a pipette when drawn up into it. Non-nucleated amebas, however, shortly after the removal of the nucleus, are but slightly if at all attached to the slide and never stick to the sides of a pipette.

The enucleated cell may move, respire, digest, respond to stimuli and exhibit any activity which is dependent solely upon catabolic or destructive processes of protoplasm. The group of phenomena which it never shows are those of growth and of regeneration and division. The phenomena of growth are essentially phenomena of organic synthesis, and the dependence of growth upon the nucleus implies the dependence of organic synthesis upon the nucleus.

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⁴ The configuration of atoms: C-N-C, so characteristic of the nucleic acid molecule, is also the configuration which occurs in the protein molecule at the points where the amino acids are joined. This fact may be not without significance.

VITA

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